HISTAMINE IN DELAYED SKIN REACTIONS

Fluorometric Determinations on Patch Tests*

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Since the demonstration by Dragstedt and Gebauer-Fuelnegg (1) of histamine release in the tissues of the sensitized animal on contact with antigen, several investigators have published experimental work on histamine in immediate allergic reactions of the skin (2, 3, 4, 5). Histamine release has also been demonstrated in the skin of patients with urticaria (6, 7, 8). The delayed type of hypersensitivity reaction, however, is usually considered to be independent of histamine alterations, and, until recently, very little attention has been given to this subject. Also the work of Inderbitzin (4) and Fisher and Cooke (9), who found a marked rise in skin histamine in the experimental dinitrochlorobenzene contact dermatitis of the guinea pig, has not been followed up.

The present study was undertaken because no investigations appear to disclose the changes in histamine content in skin in delayed skin reactions in man. This paper reports on analyses of the histamine content in positive patch tests and normal skin of patients with contact dermatitis. Besides, a series of skin samples from induced primary irritant dermatitis, were examined.

PROCEDURE

Patch testing was performed on patients with contact dermatitis by the method developed by Bonnevie (10). Adhesive tape was used for fixation of the test substances. Pieces of linen about 0.5 by 0.5 cm in size were placed in the middle of the adhesive tape. The test substances were applied to the linen. For test substances comprised of volatile fluids (as white spirit of petroleum, which was used to produce primary irritant reactions), a layer of plastic covered aluminum foil was placed between the adhesive tape and the linen. The adhesive tape was applied to the back of the patient or to the lateral surface of the upper thigh. In two cases the upper arm was used as test area. The patches were removed after 24 or 48 hours and the results read 30 minutes later. In some instances of positive reaction after 48 hours, new applications were made with the same substances, and the patches were removed after 3 or 6 hours for biopsy study.

The primary irritant reactions produced with white spirit of petroleum appeared as erythema and edema of the same extent and shape as the linen patch within 48 hours in all cases.

For the allergic reactions the following substances were used:

potassium dichromate	0.1 per cent aqueous
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mercuric chloride	0.1 per cent aqueous
mercuric chloride	0.01 per cent aqueous
nickel sulfate	1 per cent aqueous
formaldehyde	1 per cent aqueous
paraphenylenediamine	2 per cent vaseline
balsam of Peru	10 per cent vaseline
rubber, vulcanised	pure
epoxy paste	pure
neoprene glue	pure
kerodex 51	pure

Only patch tests which, after 24 or 48 hours, showed erythema, infiltration and vesicular response extending outside the linen patch were used.

After the agents were removed and the reactions read, samples of approximately 30 mg of skin were obtained from the test sites by punch biopsy excision. Normal skin was taken for control from symmetrical areas. The subcutaneous fat was removed, and, if not determined immediately, the skin specimens were frozen down at −20° C. Defatting was carried out by shaking the minced samples twice with 15 ml of ether for one hour. The defatted tissue was then lyophilized for 48 hours at room temperature under a pressure of 2 mm Hg. The defatted and dried skin samples were homogenized in 5 ml of 0.4 N perchloric acid using a motor-driven glass homogenizer. The homogenate was allowed to stand for 10 minutes and then centrifuged. A 4 ml aliquot of the supernatant was transferred to a 25 ml glass-stoppered shaking tube for histamine determination by the spectrofluorometric method of assay (11, 12).

To the shaking tube was added 0.5 ml of 5 N NaOH, 1.5 g of solid NaCl and 10 ml of butanol. The tube was shaken for 5 minutes. After centrifugation, the aqueous phase was removed by aspiration. The butanol phase was shaken for one

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TABLE I

Alterations in water content and skin histamine in allergic patch tests and induced primary irritant reactions

Type of Reaction	Number of Persons Tested	Time of Application	Average Increase in Water Content	Range of Changes in Water Content	Average Change in Skin Histamine	Range of Changes in Skin Histamine	Average Change in Skin Histamine	Range of Changes in Skin Histamine
			per cent	*per cent*	*μg/g*	*μg/g*	*per cent*	*per cent*
Allergic	14	3 hours	0.9	−2.4 to +6.5	+1.0	−4.7 to +8.8	+4.6	−22 to +51
Allergic	7	6 hours	2.6	+0.2 to +5.4	−0.7	−4.8 to +39.0	+5.7	−18 to +42
Allergic	8	24 hours	5.8	+3.1 to +9.9	+23.3	−0.7 to +61.4	+129.3	−3 to +574
Allergic	11	48 hours	5.1	+2.3 to +8.4	+34.4	+8.5 to +84.2	+133.9	+29 to +265
Allergic	6	7 days	2.2	+0.1 to +3.8	+2.7	−5.6 to +11.8	+16.6	−15 to +67
Primary irritant	18	48 hours	5.5	+1.3 to +10.6	+1.0	−12.1 to +16.0	+7.6	−43 to +159

minute with 5 ml NaCl-saturated 0.1 N NaOH as a wash. After another centrifugation an 8 ml aliquot of the butanol was transferred to a 40 ml glass-stoppered shaking tube containing 15 ml heptane and 4.5 ml 0.1 N HCl. After shaking for one minute the tube was centrifuged and 2 ml of the aqueous phase was assayed fluorometrically for histamine. Another 2 ml were used to obtain the blank value of reagents plus skin.

The 2 ml aliquot was transferred to a test tube containing 0.4 ml of 1 N NaOH followed by addition of 0.1 ml 1 per cent ortho-phthalaldehyde in methanol. These were mixed immediately, and after 3½ minutes ±½ minute the fluorophore was stabilized by the addition of 0.2 ml 3 N HCl. The fluorescence was then measured in a spectrofluorometer at 450 mμ using an excitation wave length of 369 mμ. The fluorescence blank of reagents plus skin were obtained by omission of the condensation step. This was accomplished by reversing the order of addition of ortho-phthalaldehyde and 3 N HCl.

RESULTS

In 14 patients skin histamine was determined three hours after the application of the sensitizing agent. In 7 patients, six hours after application was chosen for the reading time. In neither of these groups was it possible to find any significant changes in skin histamine, although in the last group mentioned an edema was detectable (table 1).

After 24 hours, however, a rise in skin histamine levels of the patch tests was found (fig. 1). Of 8 patients examined only two showed no significant change. The average rise in skin histamine for this group was 129.3 per cent or 23.3 μg/g ($p < 1$ per cent).

After 48 hours all of the patch test samples exhibited higher histamine values than the corresponding normal skin (Fig. 1). In one patient sensitive to paraphenylenediamine the increase in skin histamine was extremely high (364.2 μg/g or 500 per cent). To make sure that this rise did not originate from the applied test substance, paraphenylenediamine was tested with a view to a possible reaction with ortho-phthalaldehyde producing a fluorophore similar to the condensation product of histamine and ortho-phthalaldehyde. But the paraphenylenediamine was found not to interfere with the assay. For statistical reasons, however, the results from this patient were excluded. The average rise in skin histamine for the remaining eleven patients belonging to this group was 133.9 per cent or 34.4 μg/g ($p <$ 0.1 per cent).

Six patients were examined seven days after the application of the test substances and five days after the removal of the patch. Only one patient still showed a moderately increased skin histamine (Fig. 1).

The primary irritant reactions, which were all examined 48 hours after the application of white spirit of petroleum, and all of which showed a high degree of erythema and edema, showed no significant increase in skin histamine (Fig. 1). The average skin histamine level of 18 patients examined was 19.7 μg/g for the patch tests in comparison with 18.7 μg/g for normal skin of a symmetrical area.

In order to estimate the variation of histamine in normal skin of the same area, a sample measuring 8 by 2 cm was taken from a patient operated on for lumbar disc protrusion at the hospital's department of neuro-surgery, and histamine was determined in 11 different pieces from various

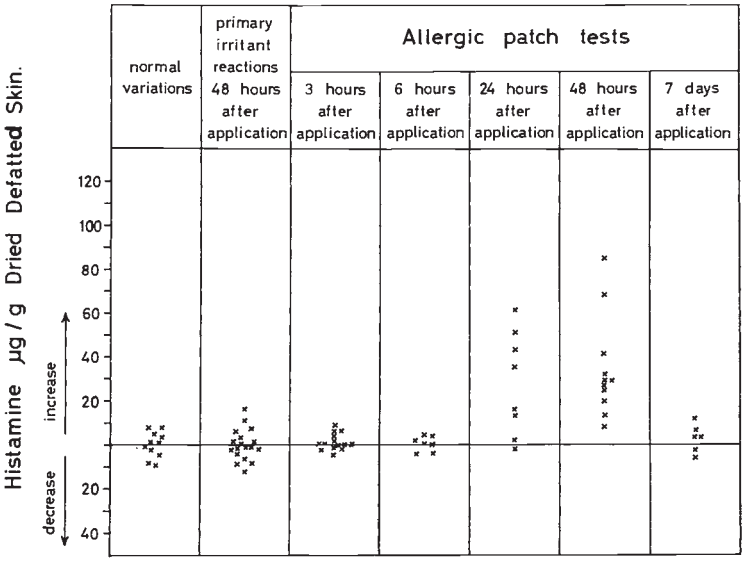

FIG. 1. Alterations in skin histamine in allergic patch tests and primary irritant reactions compared with normal variations in skin histamine from the same area.

TABLE II

Variation of skin histamine in normal skin from the same area (determinations in 11 different pieces from a skin sample measuring 8 by 2 cm)

No.	µg Histamine/g Dried and Defatted Skin
1	48.6
2	45.6
3	32.5
4	35.7
5	40.1
6	38.8
7	31.8
8	44.1
9	48.6
10	41.2
11	42.6

parts of this skin sample (table 2). These results showed a standard variation of 5.8 µg/g. The standard error of the method itself was calculated to be 4.5 µg/g, when comparing determinations of the same homogenate.

All values represent histamine base per gram defatted dried tissue.

COMMENTS

In acute allergic dermatitis, intra-epidermally located blisters characterize the histologic picture. Considerable spongiosis with intercellular and intracellular edema is a constant finding. The upper dermis shows vascular dilatation, edema and perivascular infiltration of lymphocytes and granulocytes (13). The mode of vesicle formation is still under dispute. Jadassohn (14) considers intra-epidermal edema the primary event accompanied by a migration of lymphocytes into the epidermis. Another point of view (15, 16) is that intracellular liquefaction necrosis is the primary factor and that spongiosis occurs secondarily. Miescher (17), however, states that, although he has observed primary lesions formed by the lysis of two or three squamous cells through cytoplasmic alterations in clinical cases of dermatitis, he never observed this phenomenon when carrying out patch tests with sensitizing agents, it was seen only in response to a primary irritant. This supports the idea that in delayed allergic reactions as well as in immediate allergic reactions of the skin the edema is the primary factor, while in primary irritant reactions an intracellular necrosis is the first event.

As histamine is a potent edema-producing substance, the release of which has been demonstrated in immediate allergic reactions (1, 2, 3, 4, 5), the possibility exists that the edema of the delayed allergic reaction might also be due to histamine. However, this was not demonstrated in this study. No significant differences in skin

histamine were found in the initial stages of the experimental contact dermatitis. The failure to demonstrate histamine alterations in the skin in in these stages does not, however, argue against histamine as a mediating agent. The processes may take place so slowly, that released histamine is compensate for by histamined in basophil leukocytes. These are considered to belong to the mast cell system (18, 19) and to represent a mobile source of histamine (20). Basophil leukocytes have been demonstrated in allergic patch tests by Wolf-Jürgensen (21) and Aspegren *et al* (22) in cellular contents of exudates. Their results correspond with the marked rise in skin histamine level found in the present investigation 24 and 48 hours after the application of the test substance. The absence of a similar rise in the primary irritant reactions also accord with the results of Aspegren *et al*, who found no metachromatic cells in the vesicles of the skin treated with the primary irritants cantharidin and white spirit of petroleum.

Although the present investigation gives no clear answer to the role of histamine in delayed cutaneous reactions, it indicates that histamine may have a part to play in allergic contact dermatitis. It indicates that in patch tests a significantly higher histamine level is specifically associated with allergic response in contrast to non-allergic reactions. This observation has bearing upon the differential diagnosis between allergic and primary irritant skin reactions.

SUMMARY

Analyses of skin histamine in experimental allergic contact dermatitis as well as in induced primary irritant dermatitis in patients with allergic contact dermatitis have been carried out by spectrofluorometry.

No significant alterations were found in primary irritant reactions or in the initital stages of delayed allergic reactions. After 24 and 48 hours, however, a marked rise in skin histamine was observed in the allergic patch tests.

It is suggested that histamine plays a part in allergic contact dermatitis, and that, in patch tests, a significantly higher histamine level is specifically associated with allergic response.

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